Review

Insulin resistance in hepatocytes and sinusoidal liver cells: Mechanisms and consequences

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Hepatic insulin resistance is an important underlying cause of the metabolic syndrome that manifests itself in diseases such as diabetes type II, atherosclerosis or non-alcoholic fatty liver disease (NAFLD). In this paper, we summarize comprehensively the current state of knowledge pertaining to the molecular mechanisms that lead to insulin resistance in hepatocytes and sinusoidal liver cells.

In hepatocytes, the insulin resistant state is brought about by at least one, but more likely by a combination, of the following pathological alterations: hyperglycaemia and hyperinsulinaemia, formation of advanced glycation end-products, increased free fatty acids and their metabolites, oxidative stress and altered profiles of adipocytokines. Insulin resistance in hepatocytes distorts directly glucose metabolism, especially the control over glucose output into the circulation and interferes with cell survival and proliferation, while hepatic fatty acid synthesis remains stimulated by compensatory hyperinsulinaemia, resulting in steatosis.

Very few studies have addressed insulin resistance in sinusoidal liver cells. These cells are not simply bystanders and passive witnesses of the changes affecting the hepatocytes. They are target cells that will respond to the pathological alterations occurring in the insulin resistant state. They are also effector cells that may exacerbate insulin resistance in hepatocytes by increasing oxidative stress and by secreting cytokines such as TNF and IL-6. Moreover, activation of sinusoidal endothelial cells, Kupffer cells and stellate cells will lead to chemo-attraction of inflammatory cells. Finally, activation of stellate cells will set in motion a fibrogenic response that paves the way to cirrhosis.

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Abbreviations: AGE, advanced glycation end-products; AMPK, AMP-dependent kinase; ChREBP, carbohydrate regulatory element-binding protein; ECM, extracellular matrix; ERK, extracellular regulated kinase; FAS, fatty acid synthase; FFA, free fatty acids; GLUT4, glucose transporter-4; GSK-3, glycogen synthase kinase-3; HSC, hepatic stellate cells; IKKβ, Inhibitory kappa B kinase beta; IL, interleukin; IR, insulin receptor; IRS, insulin receptor substrate; JNK, c-Jun-N-terminal kinase; KC, Kupffer cells; LPC, liver progenitor cells; MAPK, mitogen-activated protein kinase; MCD, methionine and choline-deficient; NAFLD/NASH, non-alcoholic fatty liver disease/non-alcoholic steatohepatitis; NF-κB, nuclear factor-kappa B; PEPCK, phosphoenolpyruvate carboxy-kinase; PI3K, phosphatidylinositol-3-kinase; PKB, protein kinase B; PKC, protein kinase C; PKD1, phosphoinositide-dependent protein kinase; PPAR, peroxisome proliferator-activated receptor; RAGE, receptor of advanced glycation end-products; ROS, reactive oxygen species; SEC, sinusoidal endothelial cells; SOCS, suppressor of cytokine signaling; SREBP-1c, sterol regulatory element-binding protein-1c; TNF, tumor necrosis factor α; VLDL, very low density lipoprotein.
1. Introduction

The metabolic syndrome (visceral adiposity, dyslipidaemia, hyperglycaemia and hypertension) is a cluster of metabolically related abnormalities predicting an increased risk for cardio-vascular diseases [1], type II diabetes mellitus, non-alcoholic steatohepatitis and certain cancers (Table 1). While the pathogenesis of the metabolic syndrome is not well understood, central obesity and insulin resistance are acknowledged as important causative factors [2,3]. Hypotheses relating central obesity to the metabolic syndrome focus on the concept that the adipose tissue, and in particular visceral adipose tissue, is a source of factors such as free fatty acids, reactive oxygen species, TNF and other adipocytokines, that impair insulin action in muscles and liver.

Insulin is the principal regulator of whole body glucose homeostasis, regulating glucose supply according to the needs. It promotes glucose disposal in adipose tissue and muscles, and prevents the liver from producing more glucose by inhibition of glycogenolysis and gluconeogenesis. Insulin also controls other important processes such as synthesis and storage of fat, protein synthesis, cell growth, cell proliferation, survival and differentiation. Therefore, assessment of alterations of glucose homeostasis evaluates only one aspect of insulin resistance. Importantly, organs, cell types and intracellular pathways do not present resistance to insulin action at the same time or to the same extent. Several complications or negative consequences of the insulin resistant state result from adverse impact of compensatory hyperinsulinaemia on cell types or intracellular pathways that remain, normally or partially, insulin sensitive. This is well exemplified by the polycystic ovarian syndrome or by persistent hepatic de novo lipogenesis.

The liver is an insulin sensitive organ that plays a key role in the regulation of the whole body energy homeostasis. Insulin resistance in metabolically very active hepatocytes is thus expected to have important systemic consequences. Besides this, insulin resistance, now recognised as a pathological factor in the development of non-alcoholic fatty liver disease [4], is also a determinant of disease progression in chronic viral hepatitis C and alcohol-induced liver disease [5]. These clinical observations provide further evidence that factors linked to insulin resistance exert important pathobiological effects on the liver.

The aim of this review is to summarize the alterations of insulin signaling in individual cell types constituting the liver, and to evaluate their functional consequences, at the level of the cell, the organ or the whole body, in the context of the insulin resistance syndrome.

2. Definitions and assessment of insulin resistance

Systemic insulin resistance is defined as the increased requirement for insulin to maintain glucose homeostasis (Fig. 1). Peripheral insulin resistance refers to diminished insulin-mediated uptake of glucose principally by skeletal muscle. It depends primarily on the control of GLUT4 glucose transporter expression and translocation to the plasma membrane. Hepatic insulin resistance describes impaired suppression of hepatic glucose production, which largely accounts for hyperglycaemia and glucose intolerance.

The hyperinsulinaemic-euglycaemic clamp method is the gold standard to assess insulin sensitivity in vivo [6,7]. In this technique insulin is infused continuously. The amount of glucose to be infused to maintain constant blood glucose levels is proportional to the amount of glucose taken up and metabolised by the muscle, and thus to the peripheral insulin sensitivity. Glucose tracers (radioactive or stable isotopes) are used to measure the hepatic glucose output. During the clamp, the suppression of hepatic glucose output by low doses of insulin is a measure of hepatic insulin sensitivity.

Cellular insulin resistance is defined as the alteration of the intracellular propagation of the signals evoked upon activation of the insulin receptor [8]. It is evaluated by measuring phosphorylation of intermediate proteins, changes in activity of intermediate kinases and/or modulation of target gene expression or target cellular functions in response to insulin stimulus.

3. Insulin signaling

Studies from numerous laboratories have elucidated the principal features of insulin action at the molecular

### Table 1

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<th>Metabolic syndrome definition: International Diabetes Federation [1]</th>
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<td>Central obesity</td>
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<td>Or, BMI &gt; 30 kg/m²</td>
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<td>Plus any two</td>
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<td>Raised triglycerides</td>
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<td>Reduced HDL cholesterol</td>
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<td>Raised blood pressure</td>
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<td>Raised fasting plasma glucose</td>
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* Cutoff waist circumferences for white people of European origin. Refer to [1] for cutoff values for other ethnicities.
level (reviewed in [8–12]). They are schematically represented in Fig. 2. Insulin signaling is triggered by binding of insulin to its receptor located in the plasma membrane of target cells [13]. The insulin receptor (IR) is a receptor tyrosine kinase that uses docking proteins, such as insulin receptor substrates (IRS) 1–6, Shc, Cbl, p62dok, and Gab-1, to mediate his signaling [11,14]. Tyrosine phosphorylation of IRS by insulin is a crucial

Fig. 1. (a) Insulin regulates glucose homeostasis and maintains normoglycaemia. Insulin favours glucose uptake by adipocytes and skeletal muscles and, by controlling glucose synthesis and storage, inhibits hepatic glucose output. In addition, insulin inhibits the activity of hormone-sensitive lipoprotein lipase, and thereby stimulates lipid storage in the adipose tissue. (b) In the insulin resistant state, decreased insulin sensitivity results in decreased glucose uptake by the peripheral tissues and decreased inhibition of hepatic glucose output. This concurs to hyperglycaemia and compensatory hyperinsulinaemia. The inhibition of lipoprotein lipase is reduced, leading to increased lipid storage in non-adipose tissues such as the liver and the muscles. This has significant consequences on insulin signaling in those tissues. In addition, the balance of adipocytokines is altered in fat-laden and insulin resistant adipose tissue impinging on insulin sensitivity.

Fig. 2. Schematic representation of intracellular insulin signaling. Upon insulin binding, the cytoplasmic kinase domain of the activated insulin receptor (IR) trans-phosphorylates several tyrosine residues, acting as docking sites for downstream interacting proteins. Insulin receptor interacting proteins (including insulin receptor substrates (IRS) 1–6, Shc, Cbl, p62dok, or Gab-1) are in turn phosphorylated, activating three main pathways: (1) the phosphatidylinositol-3-kinase (PI3K) pathway is mainly involved in the control of metabolic actions by insulin (glucose, lipid and protein metabolism), transcription of GLUT4, protein synthesis (via mTOR) and control of cell survival, (2) the mitogen-activated protein kinase (MAPK) pathway which mediates the mitogenic, growth and cell differentiation effects, and (3) signal transduction through the CAP/Cbl/Tc10 pathway which controls the membrane translocation of glucose transporter GLUT4, in GLUT4-expressing cells.
event in mediating insulin action, defective in most cases of insulin resistance, both in experimental models and in humans [15].

There are three major pathways emanating from the activated IRS (Fig. 2): (i) the PI3K–Akt pathway which is mainly involved in the control of metabolic actions by insulin (glucose, lipid, and protein metabolism), (ii) the MAPK pathway which mediates the mitogenic, growth and cell differentiation effects, and (iii) signal transduction through the CAP/Cbl/Tc10 pathway which controls the membrane translocation of GLUT4. In adipocytes and muscle cells, insulin uses the latter pathway to regulate glucose intake. In hepatocytes, glucose transport is mediated through GLUT2. This transporter responds to the gradient of glucose concentration across the membrane. Its expression is not regulated by insulin. The physiological control of the rate and direction of glucose fluxes across the plasma membrane of the hepatocyte depends on intracellular glucose phosphorylation/dephosphorylation balance. Insulin promotes indirectly hepatic glucose influx by stimulating glucokinase and thus the use of glucose for energy consumption, glycogen and lipid synthesis.

3.1. Control of hepatic glucose production

Upon binding of insulin to its receptor, tyrosine phosphorylation of IRS1 and 2 results in the recruitment of phosphatidylinositol-3-kinase (PI3K), which phosphorylates phosphatidylinositol [4,5] biphosphate into phosphatidylinositol [3–5] triphosphate (PIP3) [16,17]. PIP3 recruits to the membrane and activates the serine/threonine kinases PKD1 and PKB/Akt. The activation of this pathway mediates glycogen synthesis, via PKB/Akt inhibitory phosphorylation of glycogen synthase kinase 3 (GSK3), a kinase that negatively regulates glycogen synthase. It inhibits, via PKB/Akt-activation of FOXO-1, the transcription of key enzymes for gluconeogenesis: phosphoenolpyruvate carboxy-kinase (PEPCK) and glucose-6-phosphatase. Thus through activation of PI3K and PKB/Akt, and subsequent inactivation of GSK3 and activation of FOXO-1, insulin promotes storage of glucose as glycogen and inhibits glucose synthesis and glucose output.

3.2. Transcriptional control of hepatic lipogenesis

The transcription factor sterol regulatory element-binding protein (SREBP-1c) mediates most of insulin’s effects on lipogenesis, by regulating the entire program of mono-unsaturated fatty acids synthesis [18]. SREBP-1c is subjected to complex regulations [19] (Fig. 3). Several lines of evidence suggest that insulin regulates transcription, maturation and activity of SREBP-1c [20]. Classically, those pathways remain insulin sensitive. Moreover, phosphorylation of SREBP-1c by GSK3 or ERK, an intermediate of the MAPK pathway,
modulates its activity. In addition, TNF, the expression of which is increased in insulin resistant states, stimulates the maturation and the activity of SREBP-1c. Thereby, TNF participates in increased intrahepatic lipid synthesis [21] (Fig. 3).

3.3. Cell growth, proliferation and survival

Downstream of IR phosphorylation, IRS, Gab-1 and Shc activate the mitogen-activated protein kinase (MAPK) cascade via activation of the G protein Ras (Fig. 2). The MAPK pathway is associated with the mitogenic and proliferative effect of insulin via the control of the cell cycle, but it does not appear to have a major role in mediating insulin effects on hepatic glucose production or on anabolic effects of insulin [10].

The PKB/Akt pathway also participates in mediating the effect of insulin on cell growth and survival. Phosphorylated Akt promotes anti-apoptotic effects and protein synthesis. The initiation stage of protein translation is controlled by eIF2B, a guanine nucleotide exchange factor inhibited upon phosphorylation by GSK3, and protein biosynthesis is stimulated by PKB/Akt-dependent phosphorylation of mTOR [10].

4. Molecular mechanisms for insulin resistance

4.1. Underlying mechanisms

Several mechanisms, acting individually or in synergy, inhibit insulin signaling [8–12]. First, signal propagation may be altered by decreased expression (or increased degradation) of any one of the components of the insulin cascade. Increased protein expression or activation may also act as negative feedback signals. Second, proteins of the pathway may undergo post-translational modifications changing their activity. In particular, inhibitory serine phosphorylation of IRS1/2, by various kinases, alters the capacity of this protein to engage in insulin-receptor signaling [22]. Among the IRS-modifying enzymes, mounting evidence indicates that activation of stress-activated protein kinase JNK1, inhibitory kappa B kinase beta (IKKβ) and protein kinase C (PKC) is central to mediating insulin resistance in response to various stress factors such as exposure to lipid metabolites and mediators, to pro-inflammatory cytokines, to oxidative stress and to stress of the endoplasmic reticulum (ER stress) (reviewed in [23]). The third level of regulation is the interaction with inhibitory proteins. SOCS proteins are a family of suppressors of cytokine signaling induced by inflammatory cytokines, including low-grade inflammation associated to obesity. SOCS-3 seems to reduce insulin signaling by inhibition of the insulin receptor and by ubiquitin-mediated degradation of IRS1 and 2 [11,24]. Finally, increased activity of phosphatases will dephosphorylate intermediate proteins and reverse insulin action. The role of tyrosine phosphatases and PIP3 phosphatases such as Pten and SHIP in putting a break on insulin signaling is increasingly recognised [25].

4.2. Insulin resistance-inducing factors

Insulin resistance is classically associated with obesity [2]. Although epidemiological correlations are established, the cellular and molecular mechanisms are only partially unravelled. Increased visceral adiposity is associated with lipid accumulation in the liver. The latter correlates with the severity of insulin resistance [26]. Growing evidence links a chronic, low-grade inflammatory state as well as chronic oxidative stress to the co-existing conditions of obesity, insulin resistance and metabolic syndrome [27,28]. The production of adipocytokines is altered in fat-laden adipocytes. Many adipocytokines modulate insulin sensitivity and the inflammatory reaction.

4.2.1. Hyperinsulinaemia and hyperglycaemia

The most obvious clinical parameters modified in the insulin resistant state are increased glycaemia and the subsequent compensatory hyperinsulinaemia. Both hyperinsulinaemia and hyperglycaemia per se are factors that exacerbate the insulin resistant state. Hyperinsulinaemia induces downregulation of the IR at the protein level by ligand internalisation and degradation, and resistance downstream of the receptor by increased degradation of IRS1/2 and other insulin signaling molecules [23,29]. In addition, hyperinsulinaemia can damage the pancreatic β-cell and participate in reduced insulin secretion and type II diabetes (reviewed in [30]). Experiments in diabetic Zucker rats and in streptozotocin-induced type I diabetic rats at a stage of hyperglycaemia and hypoinsulinaemia suggest that hyperglycaemia reduces the efficiency of the activation step from PI3K to PKB/Akt, but does not affect the proximal part of the insulin-receptor mediated pathway [31,32].

Importantly, hyperglycaemia and hyperinsulinaemia both concur to stimulate de novo hepatic lipogenesis by activation of the transcription factors carbohydrate regulatory element-binding protein (ChREBP) and SREBP-1c, respectively [20].

4.2.2. Fatty acids

By analogy to the mechanisms of insulin resistance unravelled in muscle [8,33], the role of fatty acids or fatty acid metabolites in inducing hepatic insulin resistance has been explored. There is an inverse correlation between hepatic triglyceride stores and insulin sensitivity [26,34,35]. Recent experiments have proposed mechanistic links between intrahepatic lipids and insulin
resistance. Kim et al. generated mice in which liver-lipo-protein lipase (LPL) was over-expressed primarily in the liver. As a result of enhanced hydrolysis of triglycerides from chylomicrons and VLDL, these mice accumulated triglycerides in the liver only. Severe alterations in hepatic insulin signaling were observed, in particular the absence of tyrosine phosphorylation of IRS2 and downstream activation of PKB/Akt [36]. Samuel et al. used rats subjected to a 3-day high fat diet to stimulate hepatic fat accumulation [37]. Such a regimen did not alter peripheral insulin sensitivity, fasting plasma glucose concentrations or basal rate of hepatic glucose production. However, insulin infusion during the clamp study failed to inhibit hepatic glucose output, demonstrating hepatic insulin resistance. At the molecular level, insulin-stimulated phosphorylation of IRS1 and 2 was blunted. The proposed mechanism is the stimulation of PKC and JNK by increased intracellular fatty acid metabolites [37]. Thus, fat accumulation alone appears sufficient to induce hepatic insulin resistance. However, this resistance is not sufficient to alter basal hepatic glucose production, nor to induce glucose intolerance or peripheral insulin resistance.

4.2.3. Oxidative stress

As mentioned, hepatic steatosis and insulin resistance are intimately linked. A feature of hepatic steatosis is its association with oxidative stress leading to the induction of a stress response via JNK and NF-κB [38]. The origin of this oxidative stress is under debate. Increased activity of CYP2E1 or CYP4As may contribute to the production of reactive oxygen species [39]. Interestingly, over-expression of CYP2E1 in hepatic cell lines induces insulin resistance by decreasing tyrosine phosphorylation and increasing serine phosphorylation of IRS1/2 in response to insulin. This effect partially results from the activation of JNK and NF-κB [40]. Other authors suggest that the increased rate of β-oxidation, associated with lipid overload, generates peroxidation products, culminating in NF-κB activation [41]. This proposition is not supported by experimental evidence in animal models of steatohepatitis [42]. However, in humans, the generation of peroxidation products by enhanced β-oxidation may have pathologic significance given the chronicity of the steatotic disorder, the association with altered mitochondrial function and reduced anti-oxidant defences [43].

4.2.4. Stress of the endoplasmic reticulum

The endoplasmic reticulum (ER) is proposed as a site for sensing the metabolic stress and for its translation into inflammatory signals [23]. All the secretory and membrane proteins are folded into their secondary and tertiary structures in the ER. Stress of the ER is created from accumulation of unfolded proteins, energy and nutrient fluctuation, hypoxia, toxins and increased demand on synthetic machinery. Recent studies confirmed that in genetic and dietary models of obesity ER stress is increased in the adipose tissue and in the liver [44]. Notably, the two principal inflammatory pathways that disrupt insulin action, namely JNK and IKKβ, are linked to the unfolded protein response activated during ER stress. Subsequently, a close link between ER stress and insulin sensitivity has been demonstrated in vitro and in vivo [45].

4.2.5. Glycated proteins and advanced glycation end-products

Glucose reacts slowly (non-enzymatically) with free amino groups of proteins to form glycation products. The level of glycated haemoglobin is a widely used indicator to assess occurrence of hyperglycaemia. Insulin itself has been shown to be glycated, resulting in impaired biological activity [46]. Glycation products are further degraded to advanced glycation end-products (AGEs), a process accelerated by oxidative stress [47]. Increased AGEs and interaction with cellular receptor RAGE have been implicated in the pathogenesis of diabetic complications. During chronic hyperglycaemia, the soluble form of RAGE (sRAGE), acting as a scavenger receptor, is downregulated. This enhances the noxious consequences of AGEs [48]. In animal models, there is a positive correlation between dietary AGE/glycoxidation products and insulin sensitivity [49,50]. Methylglyoxal, the most important precursor to yield irreversible AGE, impairs insulin signaling: methylglyoxal appears to form a complex with IRS that induces conformational changes affecting the tyrosine phosphorylation and the docking function of these proteins [51].

4.2.6. Adipocytokines

The adipose tissue serves as a key site for the interaction of adipocytes with effector cells of the inflammatory and immune system and for the production of adipocytokines. These peptides have important properties as modulators of insulin sensitivity, lipid metabolism, inflammatory and immune reactions, inside the adipose tissue and more importantly on distant organs such as the liver (reviewed in [52–54]). Alterations in secretion of adipocytokines are specifically relevant in obesity, insulin resistance and the metabolic syndrome. Table 2 summarizes the main adipocytokines and their effects on liver cells.

Adiponectin is the most abundant adipocytokine in plasma. Its production decreases with adiposity and insulin resistance. It circulates in the bloodstream as multimere of full-length proteins or as cleaved proteins containing the globular domain only [55]. Adiponectin has anti-inflammatory properties, increases the sensitivity of hepatocytes to insulin-mediated inhibition of gluconeogenesis and hepatic glucose output, and regulates
hepatic FFA metabolism via suppression of lipogenesis and activation of fatty acid oxidation [54,55]. Adiponectin exerts its effects by binding to its receptors. Hepatocytes express mainly adipoR2, which binds with equal affinity the full-length and the cleaved globular form of adiponectin. Upon adiponectin binding, the receptor elicits activation of the transcription factor peroxisome proliferator-activated receptor (PPAR) α and stimulates the activity of AMP-dependent kinase (AMPK) [56]. Activation of PPARα enhances transcription of the enzymes of the fatty acid β-oxidation machinery [57] and has anti-inflammatory consequences, probably through transrepression of NF-κB [58]. The activation of AMPK, via regulation of acyl CoA-carboxylase activity and intracellular malonyl-CoA concentrations, inhibits de novo lipogenesis and favours fatty acid β-oxidation [56]. Thus, adiponectin combats intrahepatic lipid accumulation. This mechanism is largely implicated in the insulin sensitising effect of adiponectin. In NAFLD patients, serum adiponectin is negatively correlated to hepatic insulin resistance and to the amount of fat in the liver [59].

Tumor necrosis factor α (TNF) is an important pro-inflammatory cytokine that plays a central role in insulin resistance. Several, possibly cumulative, mechanisms by which TNF may impair insulin signaling have been proposed. First, TNF represses genes involved in uptake and storage of non-esterified fatty acids in the adipose tissue [60]. Those fatty acids are thus readily available for the liver, and increase the pool of intrahepatic FFA. Second, TNF activates JNK and IKKβ. This results in serin phosphorylation of IRS and inhibition of insulin signaling [11,23]. Additionally, JNK activation induces TNF, therefore representing an autocrine/paracrine loop potentiating insulin resistance. Several lines of evidence support the role of TNF/NF-κB-mediated activation of IKKβ as a mechanism for insulin resistance. Mice lacking TNF or TNF-receptors have improved insulin sensitivity in both dietary and genetic models of obesity [61]. High dose salicylate inhibits IKKβ activity [62] and reverses insulin resistance, hyperglycaemia and hyperinsulinaemia in obese and diabetic rodents [63], while heterozygous depletion of IKKβ protects against the development of insulin resistance during high fat feeding [64]. Conversely, mice with chronic hepatocellular activation of NF-κB, resulting in continuous activation of IKKβ, have insulin resistance and a diabetic phenotype [65].

### Table 2

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<tr>
<td>Vaso-active peptides</td>
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<td>Inhibitor of fibrinolysis</td>
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TNF, tumor necrosis factor; IL-6, interleukin 6; MCP-1, monocyte chemoattractant protein 1; VEGF, vascular endothelial growth factor; ASP, acylation stimulating protein; PAI-1, plasminogen activator inhibitor-1.
activity [54,55]. In conditions of obesity, high TNF and low adiponectin cooperate to the development of insulin resistance.

Interleukin-6: In non-inflammatory conditions, one-third of circulating IL-6 originates from adipose tissue. Circulating IL-6 is strongly associated with obesity and is a predictor of development of type II diabetes. IL-6 is an inhibitor of insulin signaling in isolated hepatocytes and in the liver of experimental animals [67,69]. In models of genetic or diet-induced obesity, injections of IL-6 neutralizing antibodies allow normalisation of IR phosphorylation in response to insulin and increase insulin-mediated suppression of hepatic glucose output [70]. This improvement of insulin signaling is restricted to the liver. Glucose uptake and insulin resistance in muscle and adipose tissue are not affected. It remains to be confirmed whether IL-6-dependent transcriptional activation of SOCS-3 is responsible for the inhibition of the IR.

Leptin, produced by adipose tissue, may be seen as an anti-steatotic hormone protecting non-adipose tissue from fat accumulation and lipotoxicity [71]. Interestingly, in obesity and states of insulin resistance, the protective effects of hyperleptinaemia seem to be limited due to leptin resistance [72].

5. Insulin resistance in hepatocytes

5.1. Pivotal role of hepatic insulin resistance for hyperglycaemia, glucose intolerance and the metabolic syndrome

Hepatic insulin resistance refers to impaired suppression by insulin of glucose production by hepatocytes [9]. Failure of insulin to inhibit hepatic gluconeogenesis and glycogenolysis is to a large extent responsible for the development of fasting hyperglycaemia and persistent stimulation of insulin production by pancreatic β-cells. Animals with tissue specific deletion of the IR have been invaluable to dissect single components of insulin signaling and to demonstrate the importance of the liver for insulin resistance (reviewed in [10,73]). Mice lacking IR in hepatocytes exhibit dramatic insulin resistance, severe glucose intolerance and failure of insulin to regulate hepatic gene expression and to suppress hepatic glucose output [74]. In contrast, normal glucose and insulin levels are found in mice with a deletion of IR in skeletal muscle [75,76]. Deletion of IR in the adipose tissue is associated with low insulin levels suggesting improved insulin sensitivity [77]. When IR is simultaneously knocked down in fat and muscle, there is no change in glucose or insulin levels. Thus, hepatic insulin resistance, but not peripheral insulin resistance, is necessary to develop hyperglycaemia and glucose intolerance.

5.2. De novo lipogenesis remains stimulated by hyperinsulinaemia in the insulin resistant hepatocyte

In obesity, increased adipocyte mass and insulin resistance, especially in visceral adipose tissue, contributes to elevated plasma levels of FFAs through lipolysis. As the rate of hepatic FFA uptake is directly proportional to plasma FFA concentrations, increased lipolysis appears as the major contributor to intrahepatic lipid accumulation. In addition, de novo lipogenesis is activated by glucose and hyperinsulinaemia through activation of ChREBP, SREBP-1c [20], and by low-grade inflammation and increased TNF (Fig. 3). Increased de novo lipogenesis in hepatocytes is indeed observed in NAFLD patients with hepatic insulin resistance. While in normal subjects, the contribution of hepatic de novo lipogenesis to the pool of hepatic fatty acids is less than 5%, it increases up to 25% in NAFLD patients [4]. Thus, fat accumulation in the hepatocytes is a direct consequence of insulin resistance in the adipose tissue and of hyperinsulinaemia. Hepatic insulin resistance contributes indirectly to hepatic steatosis by increasing glucose levels and worsening hyperinsulinaemia.

5.3. Consequences of insulin resistance for the control of survival and proliferation of hepatocytes

Insulin is a co-mitogen for numerous cell types. The MAPK pathway is associated with the proliferative effects of insulin (Fig. 2). Pharmacological inhibitors and dominant negative mutants of intermediate proteins of this cascade reduce insulin-mediated cell growth but have little impact on metabolic effects [10]. Activation by insulin of the PI3K/Akt/mTor pathway controls protein synthesis which is of paramount importance during cell proliferation [78].

As demonstrated experimentally, insulin signaling in hepatocytes is required to maintain hepatic function and to allow liver regeneration [74,79]. Insulin may be necessary to meet metabolic demands imposed by this energy consuming process. Additionally, competent hepatic insulin signaling may be pivotal for normal function/activation of pathways controlling cell cycle and cell differentiation. In hypoinsulinaemic streptozotocin treated rats, normal liver regeneration is observed at the expense of an adaptive increase of insulin sensitivity of hepatocytes [80]. Conversely, constitutive hepatic insulin hypersensitivity in mice with a hepatocyte-specific Pten deficiency is associated with enhanced proliferation of hepatocytes and hepatocellular carcinoma. Pten is a phosphatase with a main substrate PIP3. When Pten is deficient, the half-life of PIP3 is increased and PKB/Akt and MAPK are thereby constitutively activated [81].
Several groups have explored the consequences of fatty liver on hepatic cell proliferation. The restoration of liver mass after partial hepatectomy is near to normal in models of hepatic steatosis due to impaired VLDL export, decreased β-oxidation or in MCD diet-induced steatohepatitis (reviewed in [82]). By contrast, liver regeneration is impaired in rodents with disrupted leptin signaling [83], or in rodents fed a high fat diet [84]; both being associated with insulin resistance.

Although insulin clearly participates in the control of cell cycle and cell survival pathways in hepatocytes, the effects of intrahepatic insulin resistance on proliferation and apoptosis, and during the wound healing process remain to be explored. This has clinical relevance since increased susceptibility to apoptosis coupled to impaired regeneration with failure to replace damaged hepatocytes could participate in the liver pathology associated with the metabolic syndrome.

6. Consequences of insulin resistance for liver progenitor cells

Liver progenitor cells (LPCs) are bipotent cells located in terminal branches of the bile ductules and the canals of Hering. LPCs give rise to hepatocytes and to bile duct epithelial cells. In adult wild type mice, LPCs are scarce. By contrast, in the liver of obese and insulin resistant ob/ob mice, Yang et al. demonstrated the expansion of progenitor cells [85]. This phenomenon has also been described in the liver of patients with NASH [86]. The significance of proliferation of progenitor cells is still debated but might represent an adaptative response of the organ in the face of chronic cell loss and replicative senescence of mature hepatocytes [85,86]. Further studies are needed to analyse whether insulin itself, intracellular insulin resistance or the changes in cellular environment associated with the insulin resistant state influence the behaviour of LPCs.

7. Insulin resistance in sinusoidal cells

Besides parenchymal cells, the liver also contains sinusoidal cells (around 35% of total liver cells). Those cells exert important functions in liver morphology, function, defence and wound healing. Kupffer cells (KC) are liver-resident macrophages, with key function in innate immunity and in parenchymal inflammation [87]. Sinusoidal endothelial cells (SEC) form the fenestrated endothelium in the liver parenchyma. These scavenger cells are able to secrete a large array of cytokines or modulators of matrix homeostasis with paracrine or systemic effects [88]. Hepatic stellate cells (HSC) are situated in the space of Disse, lining SEC. They exert important functions such as the storage and homeostasis of retinoids, the synthesis and remodelling of extracellular matrix, the regulation of vascular tone of the sinusoids and the propagation of neural signals [89]. Upon liver injury of different origin, HSC are activated and change to proliferative, fibrogenic and contractile myofibroblast-like cells [90]. Those are the main effector cells of hepatic fibrosis.

7.1. Insulin responsiveness and insulin resistance in sinusoidal cells

The literature provides scarce information regarding insulin sensitivity of sinusoidal cells and as to whether hepatic sinusoidal cells develop insulin resistance.

The expression of the insulin receptor on KC is still controversial. SEC carry the insulin receptor. They bind and take up insulin with a high binding affinity, and participate in the hepatic clearance of insulin [91]. The absence of alteration of glucose homeostasis in mice carrying a specific deletion of the insulin receptor in vascular endothelial cells (including SEC) suggests that this cell type participates marginally in the control of whole body glucose balance. This deletion however protects against neo-vascularization under conditions of relative hypoxia and may also have effects on vascular haemodynamics [10,73]. The importance of competent insulin signaling in SEC for liver function and wound healing during pathological insults has not been explored.

The effect of insulin on HSC is unclear. In some experimental conditions, insulin has been shown to stimulate the MAPK and PI3K signal transduction pathways and cell proliferation [92,93]. In our hands, high insulin concentrations have no direct effect on proliferation, activation or collagen mRNA synthesis of human activated HSC (Durnez et al. submitted). Recently, Tsukamoto’s group has demonstrated that exposure of quiescent rat primary HSC to insulin induced the phosphorylation of IR, IRS1 and Akt, and stimulated glucose uptake. In activated stellate cells, IR and IRS were hyperphosphorylated. Exposure to insulin failed to further activate the IRS/Akt pathway or to induce glucose uptake [94]. These data suggest that the degree of insulin sensitivity is dependent on the state of activation of HSC.

7.2. Reactions of sinusoidal cells to insulin resistance-inducing factors

The second question of importance is how the hepatic sinusoidal cells react to the many consequences of insulin resistance.

All the sequelae of systemic insulin resistance (hyperglycaemia, hyperinsulinaemia, increased fatty acids, altered adipocytokine profile, intra- and extracellular AGE, etc.) contribute to arterial endothelial dysfunction [95]. Under the influence of these pathological factors,
endothelial cells undergo dramatic functional alterations [96]. Whether similar changes occur in venous, capillary or sinusoidal endothelial cells of the liver remains to be investigated.

7.2.1. Effects of increased glucose concentrations

A role for hyperglycaemia in the activation of HSC and in the pathogenesis of fibrogenesis has been proposed. Experimental evidence suggests that connective tissue growth factor (CTGF), a known fibrogenic factor, could be involved: high glucose (5–30 mM) or high insulin (20 UI/mL) concentrations stimulated CTGF mRNA and protein synthesis in rat HSC [97] at levels able to enhance significantly collagen expression, proliferation and migration of HSC [98].

7.2.2. AGEs, oxidative stress and hepatic sinusoidal cells

Up to 60% of total liver AGEs, generated during conditions of hyperglycaemia, are taken up by SEC and 20% by KC [99]. This uptake is dependent on scavenger receptors CD36, SR1 and SR2. Several reports show that AGEs have an important impact on cytokine release and oxidative stress leading to vascular complications and inflammatory reaction [100]. The influence of AGEs on liver sinusoidal endothelial cells and resident macrophages has not been explored yet.

HSC are the only liver cell type to express the specific AGE receptor RAGE. Its expression is increased in activated HSC, and is modulated by TGFβ1 expression and release by KC or SEC [109,115,116] is proposed as indirect mechanism by which leptin influences fibrogenesis. In the metabolic syndrome, circulating levels of leptin are generally elevated, but it appears more and more that the effects of hyperleptinaemia are largely prevented by the development of a state of leptin resistance. Whether resistance to leptin action occurs in hepatic sinusoidal cells is unknown.

Adiponectin has anti-inflammatory properties, by direct signaling and by opposing the synthesis, the release and the effects of TNF from macrophages within the adipose tissue and from KC. In KC, adiponectin reduces LPS-stimulated ROS production, TNF expression and enhances the release of anti-inflammatory IL-10 [117,118]. Conversely, LPS and inflammatory cytokines induce adiponectin expression in spleen and peritoneal macrophages. Whether adiponectin production by activated KC may represent a mechanism to control the intensity of hepatic inflammatory reaction and hepatic insulin sensitivity remains to be addressed.

Mice lacking adiponectin are exquisitely sensitive to hepatic fibrosis while supra-physiological levels of adiponectin prevent CCL4-induced fibrosis in wild type mice [119]. Ding et al. have found expression of adiponectin receptors AdipoR1 and R2 on cell membranes of primary rat HSC, both at the quiescent and activated state [120]. Adiponectin prevents activation, proliferation and migration of quiescent HSC [119,120]. When applied on activated cells, adiponectin induces apoptosis [120] suggesting that adiponectin may counteract fibrosis by eliminating fibrogenic effector cells. Hydropodinectinaemia such as associated to the metabolic syndrome may therefore enhance fibrogenesis. However, adiponectin activity strongly depends upon metabolic milieu, inflammatory pattern and receptor expression. Adiponectin concentrations in the plasma and liver do not always run parallel.

In obese and/or diabetic subjects increased serum levels of TNF and IL-6 originate largely from the adipose tissue. However, KC are capable of releasing, among other factors, large quantities of TNF and IL-6 directly in contact with liver cells. Those influence hepatic inflammation and fibrogenesis [121,122]. They may also act on hepatocytes to worsen insulin resistance and fatty liver.

7.3. Sinusoidal cells as actors of insulin resistance?

The last important question concerns the role of hepatic sinusoidal cells in the induction of intrahepatic and
systemic insulin resistance (Fig. 4). The adipose tissue has attracted a lot of attention as a pathogenic site of obesity-induced insulin resistance, because of the metabolic alterations, the change in adipocytokine production and the inflammatory state of this organ. As underlined by Shoelson et al.[123], the fatty liver, associated with obesity and insulin resistance, resembles the adipose tissue. As in adipose tissue, fat-laden metabolic cells (the hepatocytes) are in close proximity to macrophages (the Kupffer cells) and are surrounded by a vast network of vascular structures (the hepatic sinusoids). NASH is the hepatic complication of the metabolic syndrome. Hepatic inflammation and fibrosis may result from the exposure of the (fatty) liver to metabolic and pro-inflammatory mediators, produced by visceral fat and drained by the portal circulation. It is however also plausible that steatosis may induce a low-grade inflammatory response, similar to the adipose tissue inflammation that follows adipocyte lipid accumulation. The liver is densely (and permanently) populated by professional cytokine producing Kupffer cells, and other immune cells. Inflammatory cells may influence systemic and intrahepatic insulin sensitivity as demonstrated in mice with genetic modulation of the IKK$\beta$ system in myeloid cells [27]. Moreover, adoptive transfer of NKT cells, which are functionally and numerically deficient in the liver of obese, diabetic and severely steatotic ob/ob mice, improves fatty liver and glucose tolerance [124]. This reinforces the concept that hepatic non-parenchymal cells may be effectors of insulin resistance and metabolic syndrome.

Thus, hyperglycaemia, AGES, oxidative stress, and altered adipocytokine balance are all risk factors for intrahepatic inflammation and fibrosis. Those factors activate cells of the sinusoids to produce large quantities of cytokines. Whether those cytokines released in the close vicinity of hepatocytes participate, via paracrine stimulation, in hepatic insulin resistance and in the metabolic syndrome remains to be investigated.

8. Unresolved questions and perspectives for further research

At the molecular level, insulin resistance can be acquired through multiple mechanisms, and may affect various steps in the insulin signaling cascade. This may lead to various forms of insulin resistance. As far as insulin resistance in hepatocytes is concerned, not all insulin signaling pathways are affected in the same way. The PKB/Akt pathway that controls ultimately gluconeogenesis and glycolysis is severely affected which leads to loss of control over glucose output, the cause of hyperglycaemia and compensatory hyperinsulinaemia. On the contrary, hepatic fatty acid synthesis which is largely controlled by the transcription factor SREBP-1c does not seem to be affected in the insulin resistant state. Because of the compensatory hyperinsulinaemia, it may even be stimulated.

The hepatocyte plays undoubtedly a prominent role in the development of hepatic and systemic insulin resistance, but the liver is more than hepatocytes alone. Insulin resistance in sinusoidal liver cells is largely terra incognita. How do these cells react to the pathological changes associated with insulin resistance? Are they simply bystanders that are not affected by what happens in the hepatocytes? Are these cells target cells that develop their own form of insulin resistance? Do they respond to their changing environment by secreting soluble factors that influence hepatocytes and/or recruit inflammatory cells? To what extent do these cells participate in the load of adipocytokines in the liver? Are they able to induce or modulate insulin signaling in hepatocytes? These questions require more in-depth analysis before we can give definitive answers.

Fig. 4. Intrahepatic insulin resistance: possible interactions between sinusoidal cells and hepatocytes. Hyperglycaemia, hyperinsulinaemia, increased free fatty acids (FFA) and increased intracellular fatty acids, reactive oxygen species (ROS) and advanced glycation end-products (AGEs), altered balance of adipocytokines and the low-grade inflammation, all concur and participate in intrahepatic insulin resistance. Those factors are able to alter the biology of sinusoidal cells, in ways that are only partially understood. Whether those factors also induce insulin resistance in sinusoidal cells, and in the affirmative, whether signaling modifications alter the biology of those cells have been poorly explored. Sinusoidal cells produce reactive oxygen species (ROS) and a large array of bioactive peptides. Whether those are implicated, via paracrine stimulations, in hepatic insulin resistance remains to be investigated. KC, Kupffer cells; HSC, hepatic stellate cells; SEC, sinusoidal endothelial cells.
Many issues regarding the pathogenesis of insulin resistance in liver cells remain unresolved. Further studies in animal models, in particular in transgenic mice, will be needed. Then, animal data must be verified in cohorts of well-characterized patients. Insight into the pathogenesis of insulin resistance related diseases will pave the way to new therapeutic modalities for non-alcoholic fatty liver disease.

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